



REVIEW ARTICLE

Mesenchymal stem cell therapy in heart disease[☆]

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Abstract Cardiovascular disease is among the main causes of mortality and morbidity worldwide. Despite significant advances in medical and interventional therapy, the prognosis of conditions such as ischemic heart disease is still dismal. There is thus a need to investigate new therapeutic tools, one of which is stem cell therapy. Hematopoietic stem cells are the most studied type, and the fact that their biology is relatively well understood has led to their being used in preclinical research and clinical trials. However, the results of some of these studies have been controversial, which has opened the way for studies on other cell types, such as mesenchymal stem cells. These cells have immunomodulatory properties which suggest that they have therapeutic potential in cardiology. In the present article, the authors review the state of the art regarding mesenchymal stem cells, from basic and translational research to their use in clinical trials on ischemic heart disease, heart failure and arrhythmias, and discuss possible future uses.

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Terapia celular cardíaca com células mesenquimatosas

Resumo A doença cardiovascular é uma das principais causas de mortalidade e morbilidade em todo o mundo e, apesar de os avanços na terapêutica médica e intervenção percutânea, o prognóstico de doenças como a cardiopatia isquémica e o pós-enfarte do miocárdio continuam a ser consideravelmente inferiores ao desejado. Essa situação justifica a aposta na investigação de novas ferramentas terapêuticas, como é o caso da terapia com células estaminais. O conhecimento mais vasto das células de linhagem hematopoiética levou à sua utilização numa primeira fase, tanto em investigação pré-clínica como clínica. A existência de alguns resultados contrários e de uma melhoria correspondente às expectativas iniciais abriu a caminho a outros tipos celulares com potencial terapêutico, como é o caso das células precursoras do mesênquima. Estas células têm características imunomoduladoras que lhes conferem um racional de utilidade terapêutica em várias áreas da Cardiologia. Neste artigo, os autores efetuam a revisão do estado da arte relativo à utilização de células precursoras do mesênquima, desde a sua investigação

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pré-clínica até à sua utilização em ensaios clínicos em doentes com cardiopatia isquémica, insuficiência cardíaca de várias etiologias ou disritmias. É ainda prospetivado o futuro da utilização destas células.

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Introduction

Cardiovascular disease is among the main causes of mortality and morbidity worldwide and in many areas is the leading cause of death. Despite significant advances in recent years in medical and interventional therapy, the prognosis of conditions such as ischemic heart disease following myocardial infarction (MI) is still dismal for many patients,¹ which has stimulated an intense search for new therapeutic tools.

One such tool, stem cells, has recognized potential in this area, since their ability to differentiate into mature cells means that it may become possible to regenerate structurally and functionally damaged tissue.

Hematopoietic stem cells were the first lineage to be studied in preclinical research and clinical trials. Their biology is relatively well understood following a long period of animal research and then application in practice, mainly in the field of hematology, in which their clinical value is well established. They have also been used in numerous clinical trials for cardiovascular therapy,² but the results of some of these studies have been controversial, and even those with positive results have not had as significant a clinical impact as initially hoped, which has led investigators to consider other cell types as possible therapeutic tools.

Among the most promising are mesenchymal stem cells (MSCs), due to their strong paracrine function, which gives them potential immunomodulatory effects via anti-inflammatory and antiapoptotic actions. This may enable them to counteract the pathological mechanisms involved in various diseases of the cardiovascular system; MSCs may also be able to transdifferentiate into cardiomyocytes.³

In the present article, the authors review the state of the art regarding the translational application of these cells, from preclinical research to their use in clinical trials in various areas of cardiology.

State of the art

Preclinical research

There has naturally been more preclinical than clinical research into different aspects of the use of MSCs, including their collection, treatment and application.

In vitro differentiation of MSCs into cardiomyocytes was first demonstrated by Makino et al. in 1999,⁴ while in 2004 Wang et al.⁵ demonstrated the potential of MSCs for regeneration of myocardium in a rabbit model of MI, with considerable impact on mortality (16.7% in the treatment group vs. 35% in controls, $p < 0.05$). These and other animal studies also showed the advantages of treating stem cells in different ways before application. In a 2005 study, Hattan et al.⁶ transplanted purified cardiomyocytes

differentiated from bone marrow MSCs *in vitro* into adult mouse hearts; three months later the transplanted cells had survived and were oriented in parallel to the cardiomyocytes of the recipient heart.

There is growing evidence that MSCs have multiple paracrine effects that even without cell replacement can affect cardiac remodeling, angiogenesis and cytoprotection, with significant clinical benefits. In 2007, Dai et al.⁷ showed that injecting a medium containing cell factors secreted by MSCs improved post-infarction myocardial regeneration in rats, although this was more marked when the cells themselves were also injected. In the same year Ohnishi et al.⁸ demonstrated the role of MSCs in inhibiting cardiac fibrosis through regulation of collagen synthesis by cardiac fibroblasts, as well as their effects on fibroblast proliferation.

The hostile environment of the ischemic zone resulting from MI rapidly leads to the death of many transplanted MSCs, but ischemic preconditioning of the cells can improve their survival. In a 2005 study in an *in vivo* mouse model, Tang et al.⁹ showed that preconditioning increased the survival and viability of MSCs in the ischemic heart. Research into this treatment is ongoing, including a study by Chacko et al.¹⁰ in 2010 which found improved expression of MSCs cultivated initially in normoxia followed by 24 hours of hypoxia prior to administration.

In 2006, Amado et al.¹¹ used magnetic resonance imaging to monitor myocardial repair following mesenchymal stem cell therapy in a swine model, and found regeneration of viable tissue with contractile function in a previously fibrotic area.

With regard to the delivery of the cells, safety and effectiveness are a major concern. Llano et al.¹² showed that infusion of MSCs improves distal coronary blood flow in a swine model when administered in a single 20-ml/min bolus, an advance on previous studies in which multiple administrations had led to reduced flow. Different delivery routes also vary in effectiveness, particularly in terms of immediate homing, as shown in a 2008 study by Hale et al.¹³ in which intracoronary delivery resulted in 15% of the cells being retained in an infarcted rat heart model, while none were retained following intravenous administration. In 2009 the same group¹⁴ used a collagen matrix as a means of delivery of MSCs, which increased retention of cells in damaged tissue, although with no significant improvement in myocardial function. The search for better delivery routes continues, and in 2011 Hamdi et al.¹⁵ showed excellent results with epicardial delivery of MSCs in a mouse infarction model.

Several studies have shown the benefits of transplanting MSCs in animal models of heart disease, particularly following MI, but also in non-ischemic cardiomyopathy and other cardiovascular disorders. In 2009 Umar et al.¹⁶ showed that administering MSCs in a rat model of pulmonary hypertension

influenced the pathophysiology of the disease by reducing the hypertension and thus right ventricular overload.

Coronary disease

Although few clinical trials on coronary disease have been concluded, this is the area in which there has been most research into stem cell therapy.

In 2004, Chen et al.¹⁷ showed significant improvement in left ventricular ejection fraction (LVEF) following intracoronary injection of MSCs in 69 patients 18 days after MI; the patients thus treated had a favorable clinical course, with no complications related to the therapy.

In 2005, Katritsis et al.¹⁸ presented the results of transplanting autologous MSCs and endothelial progenitor cells in eleven MI patients. There were no adverse effects related to the therapy and left ventricular function improved, while myocardial scarring assessed by scintigraphy was reduced.

In a 2009 randomized, double-blind clinical trial by Hare et al.¹⁹ of allogenic MSCs (Prochymal®, Osiris Therapeutics) administered intravenously to 60 patients after MI, treated patients had fewer arrhythmic events. Two trials are under way by the same group (at the Miller School of Medicine at the University of Miami) of MSCs in ischemic cardiomyopathy: PROMETHEUS, in which patients with indication for coronary artery bypass grafting (CABG) and LVEF of 15–50% will receive either 20×10^6 (low dose group) or 200×10^6 (high dose group) of autologous MSCs by direct intramyocardial injection, and TAC-HFT, in which patients with similar characteristics but candidates for cardiac catheterization will receive MSCs by transcatheter cardiac injection.

A 2010 trial by Yang et al.²⁰ of intracoronary transplantation of autologous MSCs in 16 patients with anterior MI showed no adverse effects and improved LVEF at six months. In the same year, in a study by Viswanathan et al.²¹ in 31 patients in whom MSCs were injected transepically in the border zone of the infarcted area during CABG, no adverse effects were observed and myocardial perfusion improved in treated patients.

Another trial currently under way is C-CURE, carried out by Bartunek's group, in patients with chronic ischemic heart disease, using cardiac progenitor cells derived from bone marrow stem cells cultured, expanded and differentiated in a cytokine cocktail. Unpublished six-month results reported in April 2011 were favorable, with improved LVEF and reduced left ventricular end-systolic volume; more detailed results are awaited.

Of the various types of mesenchymal stem cells, there has been particular interest in the adipose lineage, in view of their ready availability and the success of several preclinical studies. In the APOLLO trial, adipose-derived stem cells were administered to 14 patients 24 hours after MI. Although the aim of the trial was to assess the safety and viability of this cell type and the means of delivery, 18-month data reported in June 2011 showed an 11% reduction in infarct area and a 6% improvement in LVEF.

Heart failure

Deregulation of the renin-angiotensin-aldosterone, neuro-endocrine and inflammatory systems is known to be involved

in the pathophysiology of heart failure (HF). Available pharmacological therapies can modify these processes at various levels, but fully effective treatment of HF remains out of reach. Stem cell therapy, particularly with MSCs, with their immunomodulatory properties, thus makes sense in the treatment of HF of whatever etiology, by interrupting the chronic inflammation cascade found in the condition.

There have been few clinical trials in humans showing that stem cell therapy is an effective treatment for HF, but they do indicate that the technique is safe.

In 2006, in a randomized trial of 24 patients with non-ischemic dilated cardiomyopathy receiving standard drug therapy in which the treatment group (n=12) received intracoronary injections of MSCs and the control group (n=12) received saline, Wang et al.²² showed that plasma levels of brain natriuretic peptide (BNP) fell at three and six months, while functional capacity as assessed by the 6-minute walk test improved, although there was no effect on left ventricular function or survival. No major complications were observed, including arrhythmias or adverse side-effects of the stem cell therapy, further evidence of the safety of the technique.

In 2010, Zeinaloo et al.²³ presented a case report of intracoronary administration of autologous MSCs in a child with dilated cardiomyopathy ineligible for heart transplantation, which led to improvement in functional class, quality of life and echocardiographic parameters of left ventricular function.

Arrhythmias

Although many studies in animal models and recently in humans have shown the benefits of MSC therapy in cardiac hemodynamics, there has been little investigation of its arrhythmic and electrophysiological effects.

In 2003 Pak et al.²⁴ noted sprouting of sympathetic nerves in pig myocardium following transplantation of MSCs, which could increase the risk of arrhythmias. However, this was not corroborated by other investigators, including Amado et al.,²⁵ who in 2005 reported no increase in sudden death in pigs undergoing intramyocardial injection of MSCs compared to placebo.

The aim is that stem cells implanted into myocardium in order to repair damaged tissue will form clusters with native myocytes to form homogeneous, functional tissue. It is now known that if the implanted cells present abnormal electrophysiological properties or spontaneous electrical activity, they may be a source of electrical excitation, leading to severe arrhythmias. If the implanted cells do not combine electrically with the surviving myocytes, they may also increase the area of conduction block in the damaged myocardial tissue.

The efficacy of MSC therapy and the risk of arrhythmias depend on the number of cells transplanted and the means of delivery. The intramyocardial route tends to result in clusters of cells in areas of non-viable tissue, which will lead to heterogeneous conduction and possible conduction block, while intracoronary administration is more likely to result in homogeneous delivery and aggregation in the desired location.

Phase 1 clinical trials of transplantation of skeletal myoblasts highlighted the importance of thorough

assessment of the risk of arrhythmias before trials in humans. In a laboratory study by Chang et al. in 2006²⁶ with optical mapping of an *in vitro* coculture model, reentrant arrhythmias (the equivalent of monomorphic ventricular tachycardia) were easily induced in cocultures with over 10% of MSCs but not in those with less than 1%. This indicates that MSCs can be proarrhythmic if large numbers are injected intramyocardially, and suggests that arrhythmic risk should be carefully evaluated in MSC transplantation models.

Another area of potential application for MSCs is chronic rhythm disturbances. Most investigators have focused on their use as biological pacemakers, but there is also increasing evidence that they could be used to repair the atrioventricular node. Both of these could in theory replace implantation of electronic pacemakers.

Finally, an emerging area of interest is the transplantation of MSCs that express specific ion channels, with a view to suppressing focal arrhythmias, as an alternative to surgical or catheter ablation. As the number of cells required would be relatively low, it may be easier to bring this therapy to clinical application.

Future directions

The results of various clinical trials under way or completed support the idea that, as with other types of stem cells, MSC therapy is safe and feasible. The next step is to accumulate evidence that administration of these cells brings benefits. As an example, the encouraging results of the APOLLO trial have led to the multicenter ADVANCE trial, currently in the patient selection stage, with 370 patients, comparing different doses of adipose-derived MSCs and placebo, with the intention of confirming the favorable conclusions of APOLLO.

Pretreatment of cells is also an area of particular interest, as shown by the preliminary results of the C-CURE trial and other trials currently under way. In the MESAM II trial, patients with chronic ischemic heart disease will receive MSCs pretreated with melatonin injected into the myocardium, since preclinical studies showed that melatonin pretreatment improved survival, paracrine activity and efficiency of MSCs in mouse models.²⁷ Other possible forms of pretreatment include ischemic preconditioning, as mentioned above, and different culture media.

Genetic modification of stem cells also has considerable potential, in order to condition them to over-express particular factors that improve homing, differentiation into cardiac cells or paracrine activity. Safety is a prime concern in this context in the short, medium and long term, since genetic changes require careful monitoring of errors accumulating in the genetic code, with possible negative consequences.

Although there is much ahead to discover and clarify, current evidence indicates that MSC therapy has a promising future. The results of the considerable amount of research currently under way in this area are eagerly awaited.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors have no conflicts of interest to declare.

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